

# Haemoglobin F levels in healthy Nigerian adults

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## Summary

Sickle Cell disease is a major genetic disorder in tropical Africa; its severity is often ameliorated by the presence of high levels of HbF, which is genetically determined. HbF was assessed in healthy Nigerian adults using the two minutes alkali denaturation method of Betke. The subjects studied included twenty-six mâles and twenty-four females all with HbA this was compared with twenty heterozygotes (HbAS).

The mean HbF of the entire subject studied was  $2.5 \pm 1.7\%$  (range 0.4-12.8%). The mean value for Individuals with HbA genotype was  $2.7 \pm 3.4\%$  (range 0.4 - 12.8%). While the AS subject had a mean of  $2.4 \pm 2.2\%$  (range 0.7-8.4%). Twenty-two percent of the population studied had value greater than 3%. The high Level of HbF among healthy adults is believed to be genetic and related to the high prevalence of sickle cell disease in this sub-region. The association between HbF and high Persistence of fetal haemoglobin (HPFH) and the thalassaemias are discussed.

**Key words:** *HbF, Hereditary persistence of fetal haemoglobin, Thalassaemias, Healthy adults.*

## Résumé

La maladie de la drépanocytose est un désordre génétique majeur dans le tropique de l'Afrique, sa gravité est souvent augmentée par la présence d'un niveau élevé de HbF, qui est évalué génétiquement. HbF était évalué chez les adultes nigériens bien portant tout en utilisant la méthode de Betke de deux minutes dénaturation d'alkali. Les sujets étudiés comprennent: vingt six mâles et vingt quatre femmes tous avec HbA, ceci est comparé par rapport avec vingt hétérozygotes (HbAS).

Le moyen HbF de tous les sujets étudiés était  $2,5 \pm 1,7\%$  (tranche 0,4 – 12,8%). La valeur moyenne pour l'individu avec génotype HbA était  $2,7 \pm 3,4$  (tranche 0,4 – 12,8%) tandis que le AS sujets avaient un moyen de  $2,4 \pm 2,2\%$  (tranche 0,7 – 8,4%) vingt deux pourcentage de la population étudiée avaient des valeurs de plus de 3%. On croit que le niveau élevé de HbF parmi des adultes en bonne santé est génétique et lié au niveau élevé de la maladie de la drépanocytose dans cette sous-région. L'association entre HbF et la persistante d'hémoglobine (HPFH) et la thalassémie sont l'objet de cet étude.

## Introduction

Fetal haemoglobin (HbF) is the major haemoglobin that is produced from the eighth week of gestation until term, its level at birth varies between 60-80%, and this is in contrast to adult where HbA is the predominant haemoglobin. Haemoglobin A2 is another physiological haemoglobin that is present in all individuals while Haemoglobin Gowers and Portland are embryonic hemoglobin found up to the eighth week of gestation.

The significance of HbF is its increase in inherited disorders of the  $\beta$ -globin gene such as the haemoglobinopathies of both the structural and the quantitative type<sup>1</sup>. High levels of

HbF can also occur in some acquired disorders that are associated with acute erythroid expansion<sup>1</sup>. The use of cytotoxic drugs could also raise the level of HbF hence the use of hydroxyurea in sickle cell disease<sup>2</sup>. Pregnancy is a physiological condition known to increase HbF.

The quantitation of fetal haemoglobin is based on its resistance to denaturation by alkali. The one-minute denaturation test of Singer<sup>3</sup> or the two-minute denaturation test of Betke<sup>4</sup> are the common methods used. The method by Singer is more accurate with large amounts of HbF while the latter method is more acceptable with lower amounts of HbF. Singer's method though a denaturation method also differs from the one minute denaturation test by using aliquots of the haemoglobin solution over longer periods of time<sup>3</sup>.

Haemoglobinopathies are common inherited disorders in this environment. The association between HbF and sickle Cell Disease in Nigerian Patients is well-studied<sup>5-7</sup> with little information on HbF levels in normal subjects hence the need for this study.

## Subject and methods

### Subjects

A total of 70 Nigerian adults aged between 20 and 50 years were studied. This comprised of 50 individuals with haemoglobin A, 26 of these were males while 24 were females. HbF was also determined in twenty heterozygous (HbAS) individuals for comparison. All individuals studied were hospital workers or students who were apparently healthy. The haemoglobin genotype of all the individuals was confirmed by electrophoresis.

### Estimation of HbF by the alkali denaturation test of Betke<sup>4</sup>

0.2ml of red cell lysate was mixed with 4mls of cyanmethaemoglobin reagent (Drabkins solution) to make a cyanmethaemoglobin solution. 0.2ml of NaOH was added to 2.8mls of the cyanmethaemoglobin solution. This was mixed thoroughly and allowed to stand for exactly 2 minutes at room temperature. Two mls of saturated ammonium sulphate was added and the mixture was allowed to stand for 10 minutes. The mixture was then filtered through a Whitman No. 42 filter paper<sup>8</sup>. The standard was prepared by adding 4.3mls of potassium cyanide and potassium ferricyanide solution to 0.7ml of the cyanmethaemoglobin solution. The absorbance of both test and standard were read at 413nm using distilled water as blank and analogue spectrophotometer (Pye Unicam SP-600)<sup>9</sup>. The percentage of HbF was calculated as:

$HbF = \frac{\text{Absorbency of test} \times 25}{\text{absorbency of standard}}$

### Haemoglobin electrophoresis

An aliquot of the haemolysate was subjected to electrophoresis for 1 hour in an alkaline medium (pH 8.5) using cellu-

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**Distribution of HbF among healthy Nigerian adults**

HbF	<1%	1-3%	>3%
Subjects with HbA	7(14%)	13(60%)	13(26%)
Subjects with HbAS	6(30%)	11(55%)	3(15%)
Total	13(19%)	41(59%)	16(22%)

lose acetate paper.

**Statistical analysis**

The mean and range were used to compare HbF levels between the different groups. Further evaluation was done using the student t-test to determine the level of significance for the mean values of HbF between the males and females. Statistical significance was accepted at  $P < 0.05$  level.

**Results**

The mean HbF of all the subjects was  $2.5 \pm 1.7\%$  (range 0.4 -12.8%). The mean value for subjects with HbA was  $2.7 \pm 3.4\%$  (range 0.4 -12.8%) while the AS heterozygotes had a mean of  $2.4 \pm 2.2\%$  (0.7-8.4%). The mean HbF of the males was not significantly different from that of the females. ( $2.5 \pm 1.5\%$  and  $2.6 \pm 1.9\%$  respectively,  $P = 0.29$ ). Twenty-two Percent of the subjects studied had values greater than 3%.

**Discussion**

HbF level is known to reach adult level by one year and the value is then less than 1%<sup>11</sup>. Population surveys have shown that a percent of normal adults have HbF levels higher than the Accepted upper limit of normal<sup>14,11</sup>, when this occurs an attempt is made at explaining this phenomenon which often is hereditary<sup>8</sup>.

When HbF is raised in adult life in the absence of any haematologic manifestations, the disorder is known as hereditary persistence of fetal hemoglobin (HPFH)<sup>12</sup>. Depending on the intracellular distribution of the HbF, the HPFH could either be pancellular or heterocellular. This study shows a high percentage of adults as having elevated HbF but the distribution of HbF was not done to ascertain if this is due to HPFH. The presence of the HPFH gene in this environment is not unlikely since HPFH interacts favourably with sickle cell anemia and  $\beta$  thalassaemia, thus ameliorating the severity of these disease<sup>13</sup>. A study by Sergeant et al showed that at least one of the parents of an individual with sickle cell anemia has slightly elevated HbF level<sup>14</sup>, a high mean HbF level in a population in which the sickle cell trait is as high as 25-30% which is the case in this environment is therefore not surprising. This study has not shown a higher HbF among the subjects with the sickle cell trait compared to the subjects with HbA so the high HbF may not be attributed solely to the presence of the S gene. The reason for the high HbF level may therefore be a genetic disorder that is independent of the sickle cell gene.

The thalassaemia syndromes are inherited quantitative disorder of haemoglobin production. The  $\alpha$ +thalassaemia predominates in Africa and the Caribbean where  $\alpha$  thalassaemia is rare<sup>15, 16</sup>, the alpha thalassaemia even though in the carrier state has haematologically normal or slightly reduced indices (MCH) is not associated with a raised level of HbF and so cannot be a reason for the elevated HbF. The  $\beta$  thalassaemia on the other hand is associated with a raised HbF of about 1-3%. this occurs in 30-50% of the people with the  $\beta$  thalassaemia trait<sup>17</sup>. The Heterozygote State of this disorder is phenotypically silent with normal haematological indices; oftentimes it is associated with recurrent anemia from haemolysis and especially during

pregnancy and other stressful conditions. The Presence of a gene for alpha thalassaemia in addition to the genes for beta thalassaemia may conceivably decrease the imbalance of globin production and consequent haemolysis and result in a milder form of the disease<sup>18</sup>. The sickle cell gene also interacts with  $\beta$  thalassaemia gene to produce sickling disorders of variable severity, this interaction tend to be particularly mild in African populations because of the likelihood of the co-inheritance of a mild B+transcription mutation, although occasional severe interactions are encountered<sup>16</sup>. A raised HbA2 is diagnostic of the classic B thalassaemia trait in the presence of hypochromia and microcytosis<sup>16, 17</sup>. Hypochromia and microcytosis is a common finding in this environment but this is often attributed to iron deficiency but oftentimes it does not respond to iron therapy. Apart from attributing the hypochromia and microcytosis to iron deficiency, the recurrent haemolysis and anaemia that is also a feature of the  $\beta$  thalassaemia trait may be attributed to other inherited haemolytic states especially G6PD deficiency which occurs in 18-25% of Nigerian males. Lack of adequate diagnostic facilities will also worsen making the right diagnosis.

Another possible genetic factor that is associated with raised HbF is S $\beta$  thalassaemia, which is similar to HPFH. The hallmark of the S $\beta$  thalassaemia is a raised HbF in the presence of hypochromic red cells<sup>17</sup>. The distinction between the two conditions is not always clear-cut, heterozygotes for S $\beta$  thalassaemia have a heterogenous distribution of HbF in the peripheral blood while those with HPFH have 15-25% of HbF, normal indices and a homogenous distribution of HbF. Globin chain synthesis and DNA will often differentiate between the two.

In conclusion, this study shows a raised level of HbF in a large number of the study population. The three main possibilities highlighted include HPFH,  $\beta$  thalassaemia and S $\beta$  thalassaemia. A previous work done has put the incidence of  $\beta$  thalassaemia as 0.8%<sup>19</sup>. There is a need to assess the distribution of HbF in the red cells and quantitative HbA2 using modern techniques since the former study by Esan used starch-gel electrophoresis and a visual inspection of the A2 band. This will assist in determining the prevalence of these hereditary haematologic disorders that could modify the severity of the sickle cell disease, a prevalent genetic disorder in this environment. Determining the red cell indices using a particle counter may not be useful since it is known that certain  $\beta$  thalassaemia mutations may have normal red cell indices<sup>16</sup> and especially in an environment with a concomitant occurrence of alpha thalassaemia. DNA technology will however be useful in confirming these findings and putting the different disorders in the right perspective, but this technology is not still available in most laboratories in our environment.

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